CELL PROLIFERATION AND HISTOMORPHOLOGY OF DISTRACTION OSTEOGENESIS DURING MONOFOCAL AND BIFOCAL LENGTHENING

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Introduction:
Bifocal or double-level (two osteotomies) lengthening is considered beneficial in extensive limb lengthening procedures. By using two sites of lengthening in a given bone segment, treatment time is theoretically decreased by half that of a single or monofocal lengthening. Bone formation and consolidation during distraction osteogenesis is considered to be less favorable in distal diaphyseal locations. During bifocal lengthening, osteotomies are often made in the distal diaphysis. The purpose of this study was to evaluate the cellular kinetics and histomorphometry of distraction osteogenesis in a monofocal and bifocal tibial-lengthening model. We hypothesized that the osteotomy location and number would effect the cell proliferation and morphology of regenerate bone following a 20% tibial lengthening.

Materials and Methods:
Eight skeletally immature goats were used for this study which was with approval of the Institutional Animal Care and Research Advisory Committee. An IMEX® Circular External Fixator was applied to one tibia in all animals. Subperiosteal osteotomies were performed at the diaphyseal midshaft (monofocal) or both the proximal and distal diaphysis (bifocal). Following a 7-day latency, distraction was initiated at a rate of 0.75 mm/day at each osteotomy level. All tibiae were lengthened to 20% original length and the animals sacrificed. Twenty-four hours prior to sacrifice, bromodeoxyuridine (BrdU) was administered (70 mg/kg/iv). The tibiae were sectioned longitudinally (EXAKT®) and one-half was prepared for undecalcified processing and the other decalcified and embedded in paraffin. For histomorphometry, 30-µm slides were stained using Sanderson’s Rapid Bone Stain (Surigpath Medical Industries) and H&E. Histomorphometry was performed using a semi-automated image analysis system and custom software package (IPI, International Inc.) with nomenclature and calculations accepted by the ASBMR. For immunohistochemistry, 5-µm slides were stained with anti-BrdU antibody (Calbiochem) and quantitative analysis was performed using light microscopy and digitizing image analysis system. Goat jejunum and peripheral nerve was used as positive and negative controls respectively.

Results:
The bifocal group obtained 20% lengthening at a mean of 35 days compared with 63 days for the monofocal group. Three distinct zones were clearly represented within the regenerate calluses of all specimens. The central fibrous interzone separated the proximal and distal mineralization fronts and new bone extending longitudinally from the original cortical surfaces. Intramembranous bone formation predominated in both groups. The monofocal regenerate calluses had significantly increased total bone volume, mineral apposition rate, osteoid surfaces, and osteoblast surfaces (p<0.05) when compared with either the proximal or distal regenerate calluses of the bifocal group (Figs.1-3). However, when the total regenerate area per lengthened tibiae were considered, there were no significant differences in total bone volume or BrdU labeling between the monofocal and bifocal groups. The lamellar to woven bone ratio was greater in the monofocal compared with the bifocal specimens. Within the bifocal group, there were no significant differences between the proximal versus distal regenerate calluses for any measured parameter. Cell proliferation based on BrdU labeling was most intense at the junction of the mineralization front and fibrous interzone.

Discussion:
The bifocal regenerate calluses produced similar total bone volumes as the monofocal calluses in roughly half the time; although qualitatively, the bifocal calluses had greater proportions of woven (less mature) bone. Total bone volume in this model correlated with the distraction period, not the level or number of osteotomies. We did not identify any quantitative or qualitative differences between the proximal and distal regenerate calluses in the bifocal lengthening group. Previous studies have reported inferior mechanical strength of the distal compared with proximal regenerate calluses. Those studies evaluated regenerate tissue that had undergone consolidation, whereas we examined regenerate callus immediately following distraction. These results suggest that the initial bone formation in bifocal regenerate calluses is similar, however remodeling and corticalization of the distal callus may be inferior to more proximal locations. The increased mineral apposition rates in the monofocal specimens suggest osteoblast activity may be enhanced as distraction osteogenesis proceeds.

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